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Dietary protein restriction and glomerular permselectivity in nephrotoxic serum nephritis

JOEL NEUGARTEN, ARTHUR KOZIN, ROBERT GAYNER, ROBERT G. SCHACHT, and DAVID S. BALDWIN

Hypertension and Renal Disease Sections, Montefiore Medical Center and Albert Einstein College of Medicine, and the Veterans Administration Medical Center and New York University School of Medicine, New York City, New York, USA

Dietary protein restriction and glomerular permselectivity in nephrotoxic serum nephritis. We have previously demonstrated that long-term dietary protein restriction ameliorates proteinuria and limits glomerular structural injury in rats with nephrotoxic serum nephritis. In the present study, we examined the influence of short-term dietary protein restriction on glomerular permselectivity. As compared to nephritic rats maintained on a normal protein diet, whole kidney and single nephron hemodynamics were lower in nephritic rats subjected to dietary protein restriction of three days duration (glomerular filtration rate: 0.79 ± 0.10 vs. 1.46 ± 0.11 ml/min, $P < 0.003$; renal plasma flow rate: 2.50 ± 0.34 vs. 3.96 ± 0.38 ml/min, $P < 0.02$; glomerular capillary pressure: 44 ± 1 vs. 53 ± 1 mm Hg, $P < 0.002$; proteinuria: 77 ± 15 vs. 224 ± 14 mg/24 hr, $P < 0.01$). This was associated with a rise in afferent resistance, from 2.99 ± 0.77 to 5.45 ± 0.94 dyn · sec · cm^{-5}, NS. In nephritic rats maintained on 24% protein, fractional clearances were elevated above control values for neutral dextrans with molecular radii exceeding 50 Å but were depressed for those with molecular radii below 30 Å ($P < 0.05$). Dietary protein restriction elevated the fractional clearances of dextrans with radii <30 Å while depressing the fractional clearances of dextrans with radii >50 Å ($P < 0.05$). The proportion of glomerular filtrate permeating the shunt pathway was elevated above control values in nephritic rats on the 24% protein diet but declined in those fed the low protein diet (NSN-24%: 0.86%; NSN-6%: 0.31%; control: 0.19%). In conclusion, renal hemodynamics are impaired, proteinuria is reduced and glomerular size-selective barrier dysfunction is ameliorated by short-term dietary protein restriction in nephrotoxic serum nephritis. The reduction in proteinuria is mediated both by a decline in glomerular filtration rate and by improved glomerular size permselectivity due to reduced utilization of the shunt pathway.

It has been demonstrated in numerous models of experimental renal injury that long-term dietary protein restriction reduces proteinuria and ameliorates progressive structural damage [1–6]. Assessment of glomerular hemodynamics after institution of dietary protein restriction has been performed in several models of renal disease [3, 7–10]. Glomerular capillary pressure is consistently reduced, however, the effects on the other determinants of glomerular filtration has varied with the timing and duration of protein restriction [9, 10].

In patients with chronic glomerular disease, fractional albumin and globulin excretion decline and glomerular size permselectivity improve following short-term dietary protein restriction of 11 to 14 days duration [11, 12]. In these studies, reduction in proteinuria and improvement in permselectivity were evident prior to any possible morphologic amelioration. Similarly, when rats that had undergone subtotal nephrectomy three months earlier were subjected to a 14 day period of reduced protein intake, fractional clearances of albumin and globulin declined in the absence of improved glomerular morphology [9]. These data suggest that dietary protein restriction can directly improve glomerular size permselectivity by a mechanism independent of its protective effect on glomerular structure.

In the present study we examined the effect of short-term dietary protein restriction on renal hemodynamics, proteinuria and glomerular size permselectivity in nephritic rats. Dietary protein restriction promptly reduced proteinuria, whole kidney and single nephron filtration and plasma flow rates and glomerular capillary pressure. Glomerular size permselectivity, impaired in nephritic rats maintained on a diet containing 24% protein, was improved by dietary protein restriction as a result of a decline in the fraction of glomerular filtrate permeating the shunt pathway. Thus, reduced proteinuria following dietary protein restriction in nephrotoxic serum nephritis is mediated both by a decline in GFR and by improved glomerular size permselectivity due to reduced utilization of the shunt pathway.

Methods

Protocol 1: Dextran sieving studies

Nephrotoxic serum nephritis was induced in male Sprague-Dawley rats 14 days after unilateral nephrectomy as previously described [13]. We chose to study hypertensive nephritic rats with reduced renal mass because this model represents a close approximation of chronic glomerular disease in man. Twenty-four hour urinary protein excretion was determined by colorimetric assay (Bio-Rad Protein Assay, Bio-Rad Chemical Division, Richmond, California, USA) [14].

Seventeen nephritic rats with heavy proteinuria were selected for dextran sieving studies, divided into two groups matched for the level of proteinuria and fed a diet containing 24% protein for two weeks. Nine nephritic rats were switched to a diet containing 6% protein for three days prior to study while the remaining rats were continued on 24% protein for an

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Table 1. Composition of diets

	6% Protein	24% Protein
	g/kg	
Casein	60	240
Dextrin	438	270
Sucrose	350	235
Corn oil	70	20
Mineral mix	51.9	51.9
Vitamin mix	10	10
L cysteine	2	2

Table 2. Data obtained after feeding 24% protein diet for 14 days

	Body weight *grams*	C_{Cr} *ml/min*	U_PV *mg/24 hr*
NSN-6%	320 ± 12	0.87 ±0.13[a]	212 ± 22[b]
NSN-24%	337 ± 17	1.01 ± 0.19[a]	224 ± 14[b]
Control	381 ± 19	1.63 ± 0.26	21 ± 4

Abbreviations are: C_{Cr}: creatinine clearance, U_PV: urinary protein excretion.
[a] $P < 0.05$ vs. Control, ANOVA
[b] $P < 0.001$ vs. Control, ANOVA

additional three days. The composition of the diets administered to study animals is described in Table 1. Diets were isocaloric and had identical mineral composition, including calcium and phosphorus. A modified pair-feeding regimen was employed in which all animals received and consumed 25 grams of their respective diet daily. Eight uninephrectomized male rats fed 24% protein served as controls.

For the performance of dextran sieving studies, rats were anesthetized with intraperitoneal inactin (100 mg/kg, Promonta, Germany) and underwent endotracheal intubation, jugular vein and carotid artery cannulations, and bladder catheterization. Mean arterial blood pressure (MAP) was monitored continuously via a carotid artery catheter. Surgical fluid losses were replaced with 0.9% saline containing ^3H dextran (20 μCi) and ^{14}C inulin (8 μCi) (New England Nuclear, Boston, Massachusetts, USA). Tritiated neutral dextrans of disperse molecular radii were prepared as previously described [15]. A continuous intravenous infusion of 0.9% saline containing ^3H dextran (15 μCi/ml) and ^{14}C inulin (6 μCi/ml) was administered at a rate of 0.067 ml/min. Lissamine green transit time through the urinary catheter was determined in each animal to coordinate blood and urine collections. Blood was withdrawn continuously from the right carotid artery for 30 minutes at a rate of 0.030 ml/min utilizing a Harvard pump (Harvard Apparatus, Natick, Massachusetts, USA). A 30-minute urine collection was begun after a period of time had elapsed equal to the transit time of Lissamine green. Urine and blood samples were collected and utilized in constructing a dextran sieving curve. Plasma and urine specimens were assayed for ^{14}C inulin to determine glomerular filtration rate (GFR) and then fractionated for ^3H dextrans by gel filtration techniques using a single gel column calibrated with standard marker proteins (Ultrogel, AcA 34/AcA 44, LKB Pharmaceuticals). The activity of tritiated dextran was determined in each fraction by liquid scintillation counting (Beckman Instruments, Inc., LS7500, Fullerton, California, USA). The void volume of the column was determined with blue dextran, and the fractional volume available to the solute (K_{av}) calculated as:

$$K_{av} = (V_e - V_o)/(V_t - V_o)$$

where V_o is the void volume, V_e, the elution volume of the solute and V_t, the total volume of the column. Stokes-Einstein radii for the individual dextran fractions were calculated from K_{av} according to the method of Granath and Kvist [16]. The sieving coefficient for dextrans of a given molecular radius was determined from the ratio:

$$(\text{Urine/plasma})_{\text{Dextran}}/(\text{Urine/Plasma})_{\text{Inulin}}.$$

A computer-generated dextran sieving curve was constructed for each period and displayed graphically.

Protocol 2: Micropuncture studies

Five nephritic male rats that were fed the 24% protein diet underwent micropuncture studies for measurement of whole kidney and single nephron filtration rate (SNGFR), filtration fraction (FF), free-flow intratubular pressure (P_T), stopped-flow pressure and afferent oncotic pressure (π_A). The methods and calculations employed have been previously described in detail [15], except that single nephron filtration fraction was estimated from whole kidney filtration fraction determined from renal vein sampling. Five additional uninephrectomized nephritic male rats underwent an identical micropuncture protocol three days after being switched to the 6% protein diet.

In order to further characterize glomerular size-selective properties, we applied the heteroporous mathematical model of Deen et al to our data [17]. This model assumes that the glomerular capillary wall is comprised of a large population of cylindrical pores of uniform radius and a smaller population of transmembrane shunts which permit unhindered passage of large neutral molecules such as large molecular weight neutral dextrans. Utilizing this model, glomerular capillary wall permselectivity can be characterized by intrinsic membrane parameters: K_f, the ultrafiltration coefficient, ω_o, a parameter which determines the proportion of glomerular filtrate passing through the shunt pathway and r_o, the radius in Angstroms of the selective pores. ω_o and r_o were calculated utilizing mean values for dextran sieving coefficients and mean values for glomerular hemodynamic parameters measured in NSN-24% and NSN-6%. For control unineprectomized animals we used values for glomerular determinants previously obtained in our laboratory on 23.4% protein.

Statistical analysis

Results are expressed as mean ± SEM. Statistical analyses were performed utilizing Student's paired and unpaired *t*-tests and analysis of variance with Scheffe's correction.

Results

Dextran sieving studies

Body weight did not differ among the three groups fed a 24% protein diet for 14 days. (Table 2). Proteinuria was markedly increased and creatinine clearance reduced in both groups of nephritic rats as compared to controls (Table 2). Proteinuria declined in NSN-6 after three days of dietary protein restriction from 212 ± 22 to 77 ± 15 mg/24 hr, $P < 0.01$.

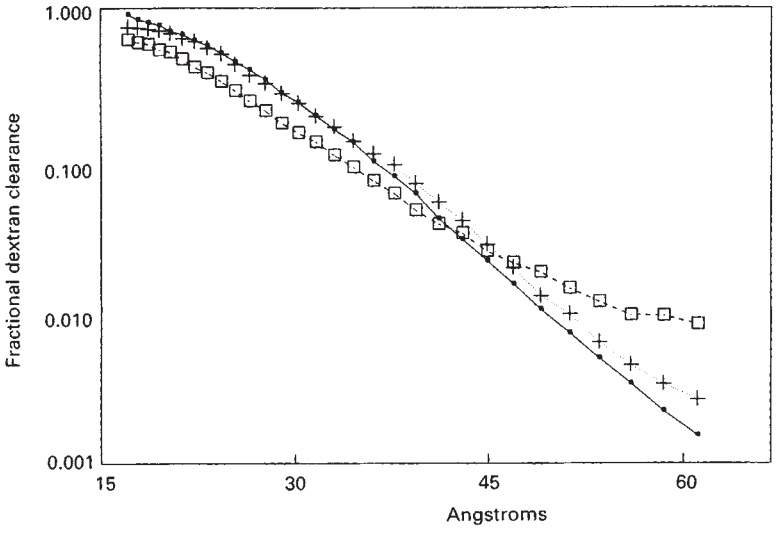

Fig. 1. *Sieving coefficients of polydisperse neutral dextrans in control and nephritic rats.* Symbols are: (--□--) NSN-24%; (··+··) NSN-6%; (-■-) control.

Table 3. Fractional dextran clearances

Molecular radius Å	Control	NSN-24%	NSN-6%
60	0.002 ± 0.0003	0.009 ± 0.003[a,b]	0.003 ± 0.001
55	0.004 ± 0.0009	0.010 ± 0.002[a,b]	0.005 ± 0.001
50	0.011 ± 0.003	0.020 ± 0.003[a]	0.014 ± 0.003
45	0.023 ± 0.005	0.027 ± 0.005	0.030 ± 0.006
40	0.066 ± 0.013	0.051 ± 0.008	0.077 ± 0.013
35	0.148 ± 0.023	0.100 ± 0.013	0.149 ± 0.018
30	0.232 ± 0.034	0.170 ± 0.020[a]	0.270 ± 0.022
25	0.519 ± 0.060	0.281 ± 0.034[a,b]	0.497 ± 0.032
20	0.838 ± 0.106	0.598 ± 0.053[a,b]	0.808 ± 0.055
15	1.094 ± 0.143	0.732 ± 0.086[a]	0.891 ± 0.063

[a] $P < 0.05$ NSN-24% vs. Control
[b] $P < 0.05$ NSN-24% vs. NSN-6%

During dextran sieving studies, mean arterial pressure did not differ among the nephritic rats (158 ± 10 mm Hg in NSN-24% vs. 163 ± 8 mm Hg in NSN-6%, NS). Inuline clearance was higher in nephritic rats fed 24% protein as compared to values obtained in nephritic rats subjected to dietary protein restriction, 1.34 ± 0.26 vs. 0.71 ± 0.14 ml/min ($P < 0.01$). As shown in Figure 1 and Table 3, in the nephritic rats maintained on 24% protein the fractional clearances of neutral dextrans were elevated above control values for dextrans with molecular radii exceeding 50 Å ($P < 0.05$), but depressed for dextrans with radii below 30 Å. Dietary protein restriction in NSN reduced the fractional clearances of dextrans with molecular radii exceeding 50 Å and increased the fractional clearances of dextrans smaller than 30 Å to values not significantly different from control values (Table 3).

Glomerular hemodynamics

In nephritic rats which underwent micropuncture studies, mean arterial pressure did not differ between those ingesting the 24% or the 6% protein diet (148 ± 2 mm Hg vs. 145 ± 4 mm Hg, NS). As shown in Table 4, animals subjected to dietary protein restriction had a lower glomerular filtration rate, renal plasma flow rate, SNGFR, and calculated glomerular capillary pressure (P_{GC}). Glomerular plasma flow rate (Q_A), π_A and K_f were also lower on the 6% protein diet, however, these reductions did not reach statistical significance (Table 4). Afferent arteriolar resistance (R_A) rose and free-flow intratubular pressure was unchanged (Table 4).

Mathematical analysis of basement membrane parameters

Using the heteroporous model of glomerular sieving proposed by Deen et al [18], we calculated pore radius and the proportion of glomerular filtrate passing through the shunt pathway. For control uninephrectomized rats we utilized hemodynamic values previously determined in our laboratory: SNGFR:57.6 nl/min, Q_A:196 nl/min, P_{GC}:48 mm Hg and K_f: 0.0897 nl · sec^{-1} · mm Hg^{-1} (unpublished data).

The calculated proportion of glomerular filtrate permeating the shunt pathway was greater in nephritic rats as compared to controls (0.86% vs. 0.19%) and declined to an intermediate value after protein restriction (0.31%). Pore radius was similar in the three groups (control: 52 Å; NSN-24%: 49 Å; NSN-6%: 51 Å).

Discussion

We have previously demonstrated that long-term dietary protein restriction reduced proteinuria and ameliorated glomerular injury in rats with a mild form of nephrotoxic serum nephritis [4]. These studies did not distinguish between functional versus structural changes as the cause of the reduced proteinuria. In the present report we studied a more severe model of nephrotoxic serum nephritis in the uninephrectomized rat characterized by hypertension, heavy proteinuria and renal insufficiency. Protein restriction of three days duration promptly reduced proteinuria, whole kidney and single nephron filtration and plasma flow rates and glomerular capillary pressure. Glomerular size permselectivity, impaired in nephritic rats maintained on a 24% protein diet, was partially restored by short-term dietary protein restriction in association with a decrease in the fraction of glomerular filtrate permeating the shunt pathway. Fractional clearance of protein declined by nearly 50% within three days after institution of a low protein diet as compared to complete remission of proteinuria following

Table 4. Glomerular hemodynamics in nephritis rats

	GFR	RPF	SNGFR	Q_A	P_{GC}	PT	A	K_f	R_A
	ml/min		*nl/min*		*mm Hg*			$nl \cdot sec^{-1} \cdot mm\ Hg^{-1}$	$dyn \cdot sec \cdot cm^{-5}$
NSN-24%	1.45 ± 0.11	3.96 ± 0.38	44.3 ± 2.1	120 ± 8	53 ± 1	11 ± 1	20.2 ± 0.7	0.0397 ± 0.0067	2.99 ± 0.77
NSN-6%	0.79 ± 0.10[b]	2.50 ± 0.34[c]	29.6 ± 2.8[b]	94 ± 12	44 ± 1[a]	10 ± 1	18.2 ± 0.9	0.0287 ± 0.0038	5.45 ± 0.94

[a] $P < 0.002$
[b] $P < 0.003$
[c] $P < 0.02$

three months of dietary protein restriction [4]. Thus, both immediate functional responses as well as long-term amelioration of progressive glomerular structural injury contribute to the reduction in proteinuria that follows dietary protein restriction. Reduced proteinuria after short-term dietary restriction is due both to a decline in GFR and improved glomerular size permselectivity resulting from a fall in the fraction of glomerular filtrate permeating the shunt pathway. Possible additional effects on glomerular charge permselectivity were not assessed in our study.

We have shown previously that impaired glomerular size permselectivity in nephrotoxic serum nephritis is a consequence of an increased fraction of glomerular filtrate permeating a non-size descriminatory shunt pathway [15]. In the present study, dietary protein restriction in NSN reduced the fractional clearance of dextrans exceeding 50 Å. The fractional clearances of smaller dextrans were increased by protein restriction. Since macromolecular sieving is determined not only by the intrinsic permeability properties of the glomerular basement membrane but also by other determinants of glomerular filtration, the observed increase in the fractional clearances of smaller dextrans after protein restriction may be attributed to declines in Q_A and ΔP, in part offset by the fall in K_f [18].

At variance with our findings, several studies suggest that glomerular basement membrane pore structure and barrier function are not influenced by manipulation of dietary protein content [19–21]. Remuzzi et al [19] evaluated proteinuria and glomerular size selectivity in rats made nephrotic with adriamycin and fed either a 20% or 35% protein diet for 21 to 45 days. Nephrotic rats fed the 20% protein diet showed reduced GFR and elevated fractional clearances of large neutral dextrans. On the higher protein diet, proteinuria increased further and GFR rose. Fractional clearances of neutral dextrans were not influenced, however, by protein feeding. Using assumed hemodynamic determinants, the calculated fraction of glomerular filtrate permeating non-size discriminatory shunt pathways and membrane pore radius were unchanged. These investigators suggested that the increase in proteinuria following high protein feeding is merely a consequence of an increased filtered load of protein. However, since proteinuria increased on average 71% on the high protein diet whereas GFR rose only 43% and serum protein concentration remained constant, this conclusion may not be justified.

Kaysen, Rosenthal and Hutchinson [20] reported a rise in GFR and a fourfold increase in the fractional clearance of albumin when normal rats were switched from an 8.5% to a 40% protein diet. However, fractional clearances of neutral dextrans did not change. Chan et al [21] examined the acute effect of a large protein meal on renal function, proteinuria and glomerular size permselectivity in patients with glomerular disease. GFR and renal plasma flow rate rose while arterial pressure and fractional clearances of albumin and IgG declined. The fractional clearance of neutral dextrans ranging in size from 36 to 54 Å declined acutely after the protein load, reflecting hemodynamic alterations rather than any change in glomerular basement membrane pore structure.

In agreement with our findings, Rosenberg et al [11] showed improved glomerular size permselectivity in patients with glomerular disease when dietary protein intake was reduced from 2 to 0.55 g/kg/day for 11 days. Fractional clearance of albumin and IgG decline while GFR and renal plasma flow rate were unchanged. The fractional clearance of neutral dextrans with molecular radii ranging from 48 to 56 Å were reduced on the restricted protein diet. Similarly, when rats with established renal injury were placed on a 6% protein diet three months after subtotal nephrectomy, fractional clearances of albumin and IgG declined [9]. Despite of a fall in P_{GC}, SNGFR and Q_A were preserved due to a rise in K_f.

In summary, we have demonstrated in nephritic rats that three days of dietary protein restriction reduces proteinuria and impairs renal hemodynamics. The decline in proteinuria is due both to the fall in GFR and improved glomerular size permselectivity. The improvement in size permselectivity occurs prior to any possible morphologic changes and results from a reduction in the proportion of glomerular filtrate permeating the shunt pathway. Possible additional effects of protein restriction on glomerular charge selectivity were not assessed.

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Reprint requests to Joel Neugarten, M.D., Renal Laboratories-Moses 605, Montefiore Medical Center, 111 East 210 Street, Bronx, New York 10467, USA.

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